

How does early snowmelt affect pollen deposition on spring wildflowers?

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Abstract

Climate change has led to increasingly warm temperatures, which has caused the earlier snowmelt. This has caused shifts in timing of some plants' growing season and flowering, as well as pollinator emergence. On the other hand, Various pollinators respond to cues such as soil temperature which does not necessarily change at the same rate as snowmelt does under climate change. As pollinators and plants often respond to these different cues for spring emergence, this can lead to a phenological mismatch. This could disrupt relationships between plants and pollinators, potentially leading to changes in pollen deposition. Pollen deposition is critical for plant reproduction. If a flower does not receive the necessary pollen then it is possible the plant will not be able to produce as many seeds. To investigate how early snowmelt affects pollen deposition in wildflowers, I will be studying the species *Claytonia lanceolata*, *Delphinium nuttallianum*, and *Geum triflorum*. These specific species of wildflowers use snowmelt as a cue for their phenology in early spring and are insect pollinated. I will collect stigmas from these species within experimentally-created early snowmelt plots and control plots. The stigmas will then be dyed and plated for pollen grain counting. This research is important because it will allow for a greater understanding of the effect of climate change-induced early snowmelt on plant-pollinator relationships.

Introduction

Climate change is progressing at an unprecedented speed, which has affected ecological communities across the globe (Rose-Person et al., 2024). In particular, snow in montane areas has begun to melt earlier due to warmer temperatures caused by climate change (Elias et al., 2021). In the Rocky Mountains, the timing of snowmelt has shifted to an earlier and earlier start date over the span of several decades (Clow, 2010). Earlier snowmelt has been observed to advance flower phenology for some plant species in montane regions because plants use snowmelt as both a cue and resource to emerge in the spring (Zona et al., 2022). Wildflowers are important to ecosystems because they play several important roles. For instance, they provide food and refuge for pollinators and herbivorous insects (Hellwig et al., 2022). They are also primary producers, making them an essential component of any ecosystem. Further, wildflowers range in size, characteristics, biology, and fulfill different niches making them all important in different ways. Changes in the timing of snowmelt could potentially impact plant communities as well as pollinator communities.

Pollinators are important to ecosystems because they play a role in ensuring the reproduction of many plants. Plant-pollinator interactions can shift in response to changes in climate (Kudo & Ida, 2013). This is possible due to the different cues each species group responds to. For example, pollinators, such as some bees, emerge when soil temperatures are warmer, not when snow begins to melt (Kudo & Ida, 2013). This could cause a temporal mismatch between the two communities because plants respond to early snowmelt, which does not necessarily shift at the same rate under climate change. This could in turn impact pollen deposition, the number of pollen grains deposition on a stigma, across various species of wildflowers.

Pollen deposition of conspecific pollen (pollen belonging to the target species) is important as it allows for sexual reproduction of flowering plants and in turn a genetically diverse seedset with high quality seeds. Therefore, this supports the stability of their population. On the other hand, if enough heterospecific pollen (pollen not belonging to the target species) is deposited onto a flower's stigma it could reduce plant reproduction due to stigma clogging or pollen allelopathy (toxicity) (Faust & Iler, 2022). Climate change could potentially increase or decrease the amount of heterospecific pollen due to the potentially altered pollinator interactions (Faust & Iler, 2022).

In Gothic, Colorado there are various wildflower species that receive several meters of snow in the winter making them wildflower species directly impacted by early spring snowmelt. They are early spring flowering species indicating that snowmelt is an important cue for their phenology. They depend on pollinators to successfully complete their life cycle, and the early snowmelt could possibly interfere with this dynamic. Studying pollen deposition within these wildflowers is key to more fully understanding the effects of early snowmelt on wildflowers and their relationship with pollinators. Therefore, the question this research will investigate is: *how does early snowmelt affect pollen deposition on spring wildflowers?* I predicted that the earliest species would have less heterospecific pollen in the early treatment than in the control treatment because there are not many other flower species in bloom so pollinators are less likely to pollinate with heterospecific pollen. The final prediction I made was that the more specialized

species would have fewer conspecific pollen in the early treatment than in the control treatment because pollinators do not typically respond to the same emergence cues. The results of this investigation and hypotheses will allow for a greater understanding of potential effects of climate change on montane wildflower communities.

Methods

This study was conducted in a subalpine meadow at the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Colorado. Data collection was conducted from mid-May to late-July 2024, which is the period right after the snow melts and these species are flowering. Plants will be monitored in eight 5 m x 5 m blocks, with each block consisting of two plots with different treatments (early snowmelt or control), summing up to 16 total plots (Figure 1). In each block, there is a control plot that receives no treatment, therefore no variables are manipulated. The other plot receives a treatment that causes the snow to melt earlier. Specifically, when the snow is around a meter deep, a black 7 m x 7 m shade cloth is suspended above the surface of the snow, causing the snow to melt about a week earlier than the control plot.

Location of Research Plots at the Rocky Mountain Biological Laboratory Research Meadow

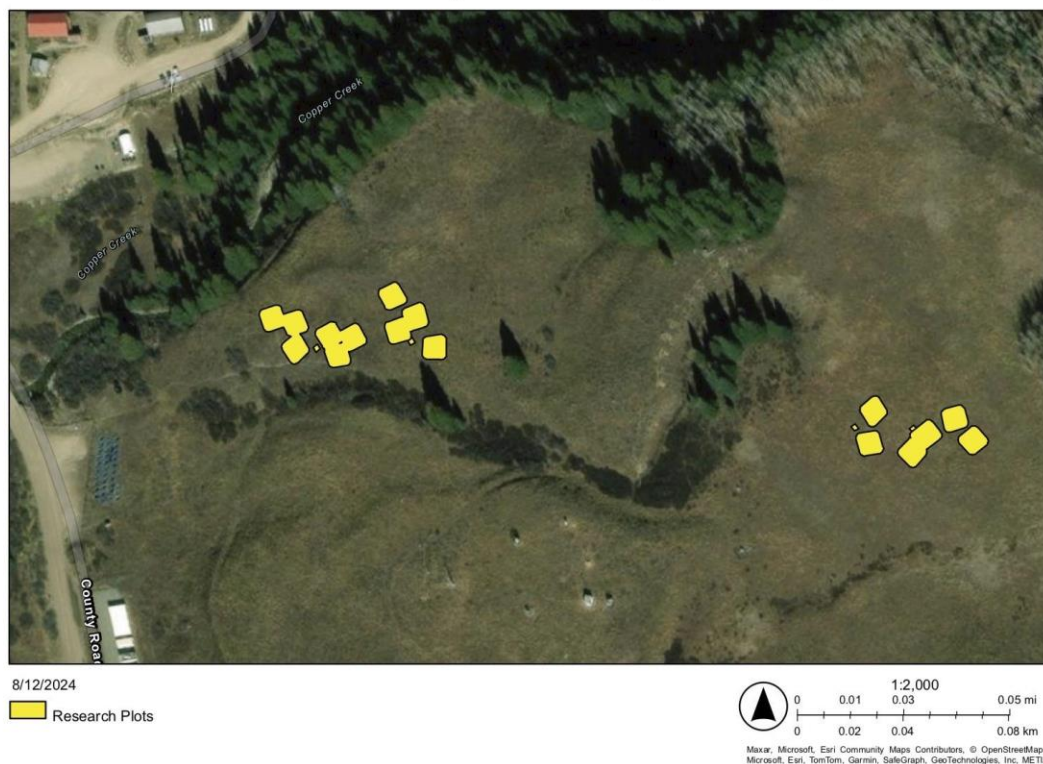


Figure 1: Map of early and control plots in the Rocky Mountain Biological Laboratory research meadow

The wildflower species that will be used in this experiment will be *Claytonia lanceolata* (Spring beauty), *Delphinium nuttallianum* (Larkspur), and *Geum triflorum* (Prairie Smoke). *Claytonia lanceolata* is the first wildflower to bloom in these plots. *Delphinium nuttallianum* and *G.triflorum* bloom later in the spring. There are 35 individuals studied per treatment for *C.*

lanceolata and *D. nuttallianum*, totaling 70 individuals per species. *G. triflorum* had many stigmas so there were only 20 individuals studied per treatment, totalling 40 individuals for this species. Combining the three species, there were 180 individuals studied in this experiment.

To determine the amount of pollen deposited on each plant individual, the number of pollen grains were counted on each collected stigma. To collect the stigmas, we used a pair of tweezers to remove it from the rest of the flower. We then placed it in a small eppendorf tube that was labeled with the plant's unique ID and treatment (early or control). It was then stored in a cooler bag to ensure that it remained fresh as we collected the rest of the stigmas.

Once the stigmas had been properly collected, they were taken to the lab and plated for viewing under the microscope. To do this, a pink fuchsin gel with a jelly-like consistency was placed on a glass microscope slide. Then it was placed over a hot plate to slowly melt. Once it was fully melted, it was carefully removed from the hot plate. We then used tweezers to extract the stigma from the eppendorf tube and placed it on the melted gel. Then a square microscope glass coverslip was placed over the gel and stigma. This was pressed down with the eraser end of a pencil to ensure the gel was spread out and the stigma slightly squashed. Next, A marker was used to write the plant ID and treatment in the space under the pink gel. Once this was completed the pollen grains on the stigmas were placed under a compound microscope at 100x magnification for viewing.

The conspecific pollen grains were identified with the use of a pollen reference collection from the RMBL herbarium and counted. We did not identify the species of heterospecific pollen grains due to time constraints and the technical skill required. All heterospecific pollen grains were grouped together and counted as well.

Analysis

I analyzed three separate response variables: the number of conspecific pollen grains, the number of heterospecific pollen grains, and the proportion of conspecific pollen grains. I used generalized linear mixed effect models (GLMMs) to understand how the early snowmelt treatment affected pollen deposition for each response for each species (total of 9 models). The predictor variables for all three models included treatment as a categorical fixed effect and plot nested within block as a random intercept term. I used a Poisson error distribution for count variables (number of pollen grains), a negative binomial error distribution if the pollen counts were overdispersed, and a binomial error distribution for the proportion of conspecific pollen, with the total number of pollen grains as a weight. All analyses were performed in R 4.4.0 (R Core Development Team 2024), using package glmmTMB for GLMMs (Brooks et al. 2017) and the tidyverse package for data manipulation (Wickham et al. 2019).

Results

The control and early snowmelt treatment had no effect on the number of conspecific pollen grains of *Claytonia lanceolata* ($X^2 = 1.41$, $df = 1$, $p = 0.24$). The two treatments also had no effect on the number of heterospecific pollen grains of *Claytonia lanceolata* ($X^2 = 0.42$, $df =$

1, $p = 0.52$) or on the proportion of conspecific pollen grains ($X^2 = 0.044$, $df = 1$, $p = 0.83$). These results are represented by box-and-whisker plots (Figure 2).

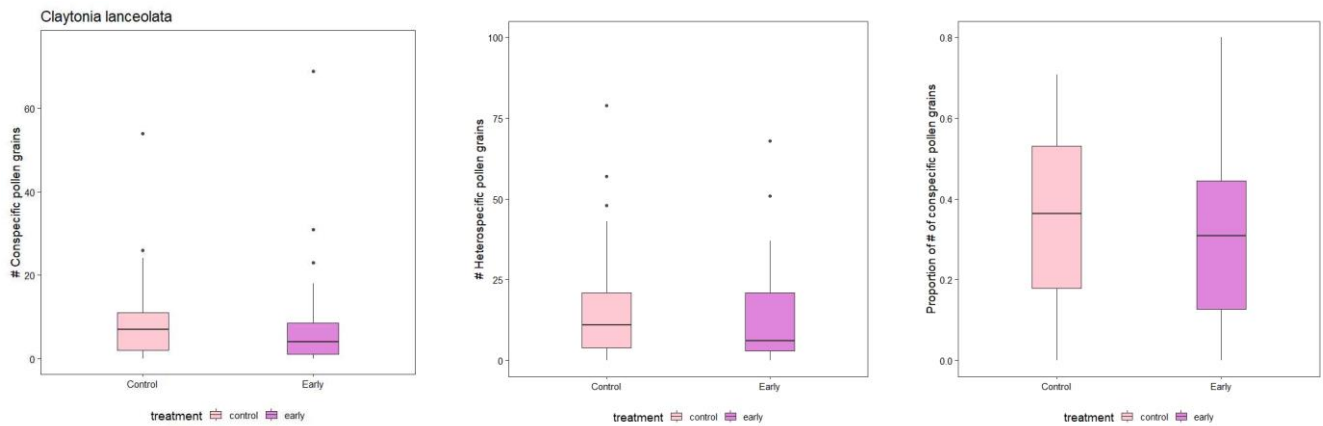


Figure 2: Three box-and-whisker plots representing *Claytonia lanceolata* pollen grain data

The control and early treatment had no effect on the number of conspecific pollen grains of *Delphinium nuttallianum* ($X^2 = 0.481$, $df = 1$, $p = 0.488$). The two treatments also had no effect on the number of heterospecific pollen grains of *Delphinium nuttallianum* ($X^2 = 1.55$, $df = 1$, $p = 0.21$) and on the proportion of conspecific pollen grains ($X^2 = 0.033$, $df = 1$, $p = 0.86$) (Figure 3).

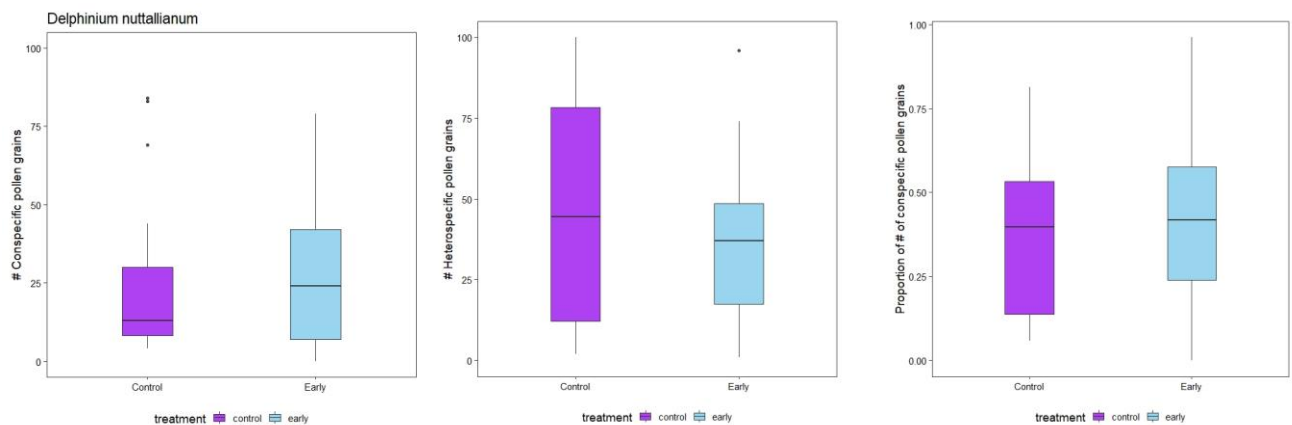


Figure 3: Three box-and-whisker plots representing *Delphinium nuttallianum* pollen grain data

The early snowmelt treatment had significantly fewer conspecific pollen grains of *Geum triflorum* than in the control treatment ($X^2 = 8.01$, $df = 1$, $p = 0.005$). The two treatments had no effect on the number of heterospecific pollen grains of *Geum triflorum* ($X^2 = 2.24$, $df = 1$, $p =$

0.134) and also on the proportion of conspecific pollen grains ($X^2 = 1.41$, $df = 1$, $p = 0.236$) (Figure 4).

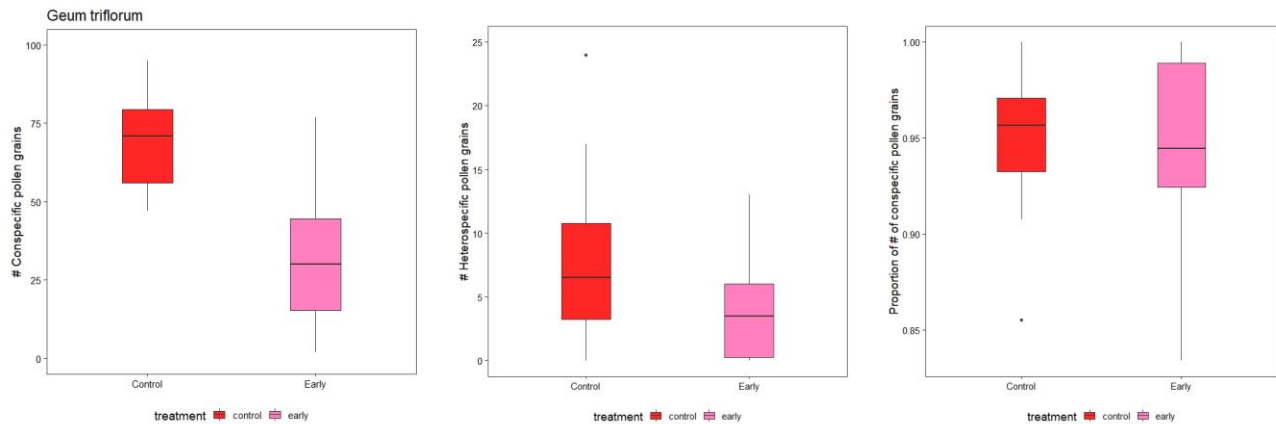


Figure 4: Three box-and-whisker plots representing *Geum triflorum* pollen grain data

Discussion

Overall, most results from this experiment did not give evidence that snowmelt affects pollen deposition. The results also did not support my hypothesis that the earliest species would have less heterospecific pollen in the early treatment than in the control treatment. The earliest flowering species observed in this experiment was *Claytonia lanceolata*. During its flowering period, there were few other surrounding flowering species making the chance of heterospecific pollen deposition low. Despite this, the results demonstrated no difference between the amount of heterospecific pollen and conspecific pollen. This could be explained by the foraging patterns of pollinators. There may not have been any other flowers closely surrounding the earliest flower species in the plots but there were other flowers in the areas outside of the plots, such as *Erythronium grandiflorum* and *Mertensia fusiformis*. If pollinators are generalized, they will pollinate whichever flower they encounter. This includes flowers that have some distance between each other. It is possible that pollinators traveled between different flowers outside the plot and then pollinated the flowers within the plot. Identifying which species the heterospecific pollen are derived from would allow for further understanding as to why there was no difference between heterospecific and conspecific pollen amounts.

There was one significant result of the nine analyses that were conducted which supported the second hypothesis that stated the more specialized species would have fewer conspecific pollen in the early treatment than in the control treatment. *Geum triflorum* is pollinated by a specialist species of bees called *Andrena transnigra*. Specialist species are defined as species that are adapted to specific conditions in an environment and need specific resources to survive (Kneitel, 2018). This indicates that *Andrena transnigra* responds to specific shifts in the environment in order for them to emerge and begin pollination. They are ground nesting bees that respond to changes in soil temperature (Kudo & Ida, 2013). If both changes in early snowmelt and soil temperature changes do not align then this would lead to a phenological mismatch between plants and pollinators. With a lesser presence of pollinators when the flowers

start blooming there may be a lesser chance for them to be pollinated, which in turn could have reproductive consequences for these early flowering species. This experiment did accelerate the rate at which snowmelt in early treatment plots which allowed for flowers to bloom earlier. However, we did not alter the surrounding landscape in which the pollinators were coming from. Future research could include manipulations on pollinator emergence as well to better reflect climate change effects on both parts of this mutualistic relationship.

Conclusion

The lack of significant evidence demonstrated that early snowmelt had no impact on any aspect of pollen grains. Despite this, the experiments conducted here could still serve as a small scale representation of the effects climate change could possibly have on pollination and plant-pollinator relationships. Future studies could include observing the reproductive success of the flowers and comparing it to the number of heterospecific and conspecific pollen grains. This could serve as another representation of how early snowmelt could affect wildflower pollen deposition. Future research could also include linking this pollen deposition data to pollinator visitation data that was collected by a previous research group. Overall, the continuation of research related to early snowmelt and pollination could further our understanding of plant-pollinator relationships and therefore contribute significantly to their protection and conservation.

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